Kidney International, Vol. 43 (1993), pp. 1015–1020

Effect of furosemide-induced hypokalemic metabolic alkalosis on renal transport enzymes

SOMCHAI EIAM-ONG, NEIL A. KURTZMAN, and SANDRA SABATINI

Departments of Internal Medicine and Physiology, Texas Tech University Health Sciences Center, Lubbock, Texas, USA

Effect of furosemide-induced hypokalemic metabolic alkalosis on renal transport enzymes. Hypokalemic metabolic alkalosis is one of the most common complications of chronic furosemide administration. In this study we examined acid-base composition and ATPase enzyme activities in medullary thick ascending limb of Henle's loop (MTAL) and collecting tubule (CCT and MCT) after seven days of chronic furosemide therapy. All of the studies were conducted in adrenal intact (AI) rats or in adrenalectomized (ADX) glucocorticoid replete rats replaced with a physiological dose of aldosterone (Aldo). Furosemide (F) was administered to each rat by mini-osmotic pump. In the AI + F group, plasma Aldo was high and obvious metabolic alkalosis occurred (HCO_3^- = 37 ± 2 mEq/liter vs. 22 ± 2 mEq/liter in controls, $P < 0.005$); activities of H-K-ATPase, H-ATPase, and Na-K-ATPase were increased approximately twofold in both CCT and MCT. In the ADX + F group (HCO_3^- = 28 ± 2 mEq/liter, $P < 0.05$ from control), H-ATPase activity was normal in CCT and it was slightly increased in MCT. CCT and MCT H-K-ATPase activities were markedly increased (~ twofold). Na-K-ATPase activity was the same as control in CCT but it was increased in MCT. In ADX + F + Vanadate (V) group which also had normal Aldo levels, acid-base changes were modest (20 ± 2 mEq/liter, NS from control); in CCT and MCT H-K-ATPase and Na-K-ATPase activities were markedly reduced, but H-ATPase activity in MCT was increased. In all three experimental groups Na-K-ATPase activity in MTAL was reduced fivefold. Hypokalemia developed in both intact and ADX animals receiving furosemide. Thus, metabolic alkalosis in chronic furosemide therapy is associated with stimulation of all three collecting tubule ATPases. The high aldosterone level likely stimulates the H-ATPase in both CCT and MCT; and in the former it also stimulates Na-K-ATPase activity. The hypokalemia probably activates H-K-ATPase in both CCT and MCT. Furosemide also appears to directly stimulate H-ATPase activity in the MCT.

Hypokalemic metabolic alkalosis is one of the most common complications of chronic furosemide (F) therapy [1–4]. The underlying mechanisms for its generation and maintenance involve aldosterone (Aldo) excess, hypokalemia, continued increased sodium delivery to the distal nephron, volume contraction or chloride depletion [1]. Furosemide inhibits Na-K-ATPase activity in the medullary thick ascending limb of Henle's loop (MTAL) secondary to the fall in sodium entry at the apical membrane [5]. The fall in sodium chloride reabsorption causes extracellular volume contraction which, in turn, activates aldosterone secretion. Increased distal tubular fluid flow, aldosterone, catecholamines, and antidiuretic hormone contribute to the increase in potassium secretion and are the major mechanisms of kaliuresis [1–4].

The collecting tubule is the final site of urinary acidification [6]. There, aldosterone and potassium influence the two renal proton ATPases, the electrogenic H-ATPase and the electroneutral H-K-ATPase. Aldosterone stimulates collecting tubule H-ATPase activity and potassium deficiency stimulates collecting tubule H-K-ATPase activity [7–12]. Aldosterone stimulates Na-K-ATPase activity in the cortical collecting tubule (CCT), while hypokalemia stimulates Na-K-ATPase activity in the medullary collecting tubule (MCT) [13–16].

We studied the effect of chronic furosemide administration on acid-base balance and on isolated tubule enzyme activity in rats. The results suggest that the interaction of aldosterone and potassium alters enzyme activity in both CCT and MCT such that both proton ATPases are stimulated, and thus maintain this form of diuretic-induced metabolic alkalosis.

Methods

Preparation of animals

These studies were performed in both adrenal intact (AI) and hormone-replete adrenalectomized (ADX) male albino Sprague-Dawley rats (150 to 200 g). In order to maintain constant drug delivery, furosemide was administered to each of the three experimental groups (described below) using an osmotic minipump (Alzet # 2002, Alza Corp, Palo Alto, California, USA) implanted subcutaneously in the intrascapular region of the animal. Furosemide was dissolved in 0.9% sodium chloride at alkaline pH (8.5 to 9.5, Tris buffer). The daily dose of furosemide administered was 3.5 mg, a dose known to produce hypokalemic metabolic alkalosis in rats [17]. In the two groups, animals received dexamethasone 1.4 μg/100 g body wt/day and aldosterone 1 μg/100 g body wt/day (via a second osmotic minipump) as maintenance glucocorticoid and mineralocorticoid replacement, respectively. Polyethylene glycol 400 was used as the diluent. All animals had free access to the usual laboratory diet and were given 0.3% NaCl to drink *ad libitum*.

The influence of furosemide on acid-base composition and H-ATPase, H-K-ATPase, Na-K-ATPase in MTAL and collecting tubule (CCT and MCT) was investigated after seven days of diuretic treatment. The animals were divided into four groups (N = 6/group):

Group 1 Control (AI paired litter mates)
Group 2 AI + F

Received for publication October 12, 1992
and in revised form December 23, 1992
Accepted for publication December 28, 1992

Table 1. Effect of chronic furosemide (F) on metabolic parameters in adrenal intact (AI) and adrenalectomized (ADX) rats (N = 6/group)

	Control	AI + F	ADX + F	ADX + F + V
P_K *mEq/liter*	4.1 ± 0.2	2.4 ± 0.2[b]	2.9 ± 0.2[b,c]	2.6 ± 0.3[b]
P_{ALDO} *ng/dl*	34.5 ± 2.5	97.7 ± 6.9[d]	5.1 ± 0.5	5.3 ± 0.3
P_{Na} *mEq/liter*	140 ± 3	137 ± 2	136 ± 3	136 ± 2
P_{Cl} *mEq/liter*	94 ± 3	79 ± 3[a]	81 ± 2[a]	83 ± 2[a]
Hct %	44.3 ± 1.0	45.0 ± 0.7	45.1 ± 1.1	45.6 ± 1.8
C_{Cr} *ml/min · 100 g body wt*	0.94 ± 0.09	0.90 ± 0.08	0.91 ± 0.09	0.89 ± 0.10
FE_{Na} %	1.5 ± 0.2	5.1 ± 0.8[b]	5.3 ± 0.4[b]	5.5 ± 0.5[b]
FE_K %	32.7 ± 2	90.2 ± 6.2[b]	87.4 ± 7.8[a]	82.6 ± 5.8[b]
FE_{Cl} %	2.7 ± 0.2	7.9 ± 0.4[b]	8.3 ± 0.6[b]	8.5 ± 0.5[b]
Body wt *g*				
Pre-treatment	149 ± 3	148 ± 2	149 ± 2	147 ± 3
Post-treatment	189 ± 3	171 ± 3[a]	170 ± 4[a]	168 ± 3[a]

Abbreviations are: P, plasma; Aldo, aldosterone; Hct, hematocrit; C_{Cr}, creatinine clearance; FE_{Na}, fractional sodium excretion; FE_K, fractional potassium excretion; FE_{Cl}, fractional chloride excretion; V, chronic vanadate administration (see **Methods** for details).

[a] $P < 0.05$ vs. control

[b] $P < 0.01$ vs. control

[c] $P < 0.05$ vs. AI + F

[d] $P < 0.01$ vs. ADX + F and vs. ADX + F + V

Group 3 ADX + F

Group 4 ADX + F + sodium orthovanadate (V)

Sodium orthovanadate (V; 5 mg/kg, intraperitoneally), was administered daily to rats for 10 days. This dose and duration of V treatment have been previously demonstrated by us to markedly inhibit H-K-ATPase activity in collecting tubule segments and cause hypokalemic distal renal tubular acidosis [18]. Sodium orthovanadate administration was begun for three days before adrenalectomy and continued for the next seven days. On the day before each experiment the rats were placed in metabolic cages, and a twenty-four hour urine was collected for measurement of sodium, potassium, chloride and creatinine as previously described [18]. On the morning of the experiment, the animals were anesthetized and a blood sample was obtained from the abdominal aorta for measurement of arterial pH and pCO_2, creatinine, electrolytes, hematocrit, and aldosterone. Plasma aldosterone concentration was determined by radioimmunoassay method (COAT-A-COUNT© DPC Los Angeles, California, USA).

Tubule microdissection and enzymatic measurements

The left renal artery was cannulated and the kidney was perfused for 15 minutes *in situ* at a rate of 0.7 ml · min^{-1} with a balanced salt solution containing 400 U/ml collagenase, 4°C, pH 7.4. The kidneys were then cut along the cortico-papillary axis and incubated in 3 ml of collagenase-albumin containing Hank's solution at 35°C for 12 minutes. The tissues were continuously bubbled with compressed air (3 psi). After incubation, the pyramids were rinsed and immediately microdissected as previously described [19]. The tubule segments were identified (MTAL, CCT and MCT) and tubule length was measured. To remove most of the extracellular potassium, nephron segments were incubated for 15 minutes at 37°C in K-free buffer [11]. The nephron segments were then subjected to a two-step hypotonic-hypothermic shock and ATPase activities were determined using γ ^{32}P-labeled ATP (37°C, 15 min, pH 7.4). H-ATPase, H-K-ATPase, and Na-K-ATPase were measured radiochemically as previously described [18, 19].

Enzyme activity is expressed as pmol/mm tubule length/hr of ATP hydrolyzed. All samples were run in triplicate or quadruplicate, and appropriate corrections were made for blanks and the spontaneous hydrolysis of ATP.

Materials

All chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA) and were of highest purity. Radiolabeled ATP was obtained from New England Nuclear (Boston, Massachusetts, USA).

Statistics

The results are expressed as the mean ± SEM. Statistical significance was assessed using the Student's t-test or analysis of covariance, where appropriate, with P values of 0.05 or less being significant.

Results

Metabolic data

Aldo levels in the AI + F group were markedly elevated (97.7 ng/dl) (Table 1). In the ADX animals on replacement via osmotic minipump, aldosterone levels averaged about 5 ng/dl (that is, ADX + F and ADX + F + V groups), values comparable to those measured in normal unstressed animals [20]. The moderately high aldosterone level seen in control animals (34.5 ng/dl) reflects the stress of surgery and anesthesia [20]. Plasma potassium in the control group was 4.1 ± 0.2 mEq/liter. Profound hypokalemia developed in all animals receiving furosemide. Serum potassium averaged 2.5 mEq/liter in the three groups ($P < 0.01$ vs. control). It should be noted that hypokalemia occurred in both the ADX + F and ADX + F + V groups, groups in which aldosterone levels were physiologically normal owing to the miniosmotic pump infusion. Plasma potassium in the AI + F group, the group having the highest aldosterone level, was significantly lower than the potassium noted in the ADX + F group (2.4 ± 0.2 mEq/liter vs. 2.9 ± 0.2 mEq/liter, respectively, $P < 0.05$). Plasma chloride was decreased in the three experimental groups receiving furosemide. As expected, the fractional excretion of sodium, potassium and chloride was increased. Neither hematocrit nor

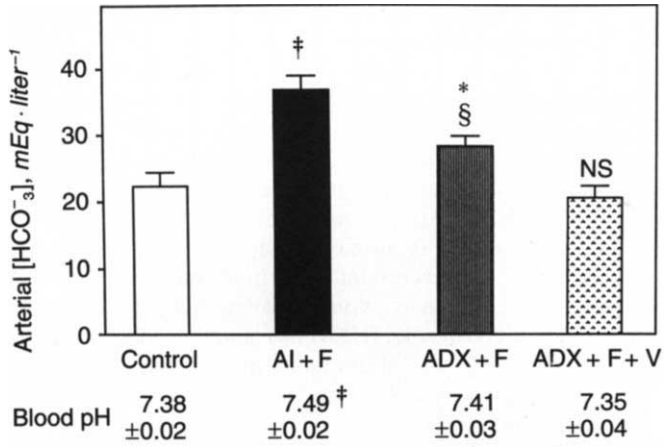

Fig. 1. *The effect of furosemide (F) on arterial blood pH and plasma bicarbonate in adrenal intact (AI) and adrenalectomized (ADX) rats;* N = *6 in each group*. Symbols are: (■) AI + F; (▨) ADX + F; (▩) ADX + F + V; (□) control. *$P < 0.05$, ‡$P < 0.005$ vs. control animals; §$P < 0.05$ vs. AI + F; NS, not significant vs. control.

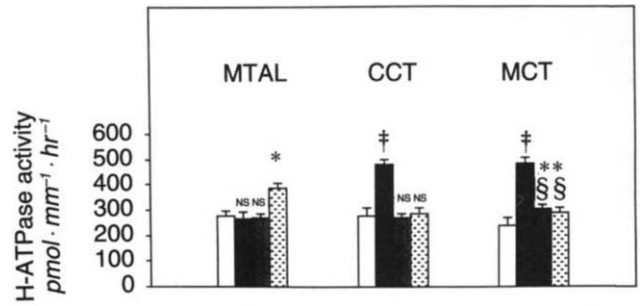

Fig. 2. *The effect of furosemide (F) on H-ATPase activity in MTAL, CCT, and MCT in adrenal intact (AI) and adrenalectomized (ADX) rats;* N = *6 in each group*. Symbols are: (■) AI + F; (▨) ADX + F; (▩) ADX + F + V; (□) control. *$P < 0.05$, ‡$P < 0.005$ vs. control animals; §$P < 0.01$ vs. AI + F (in MCT); NS = not significant vs. control.

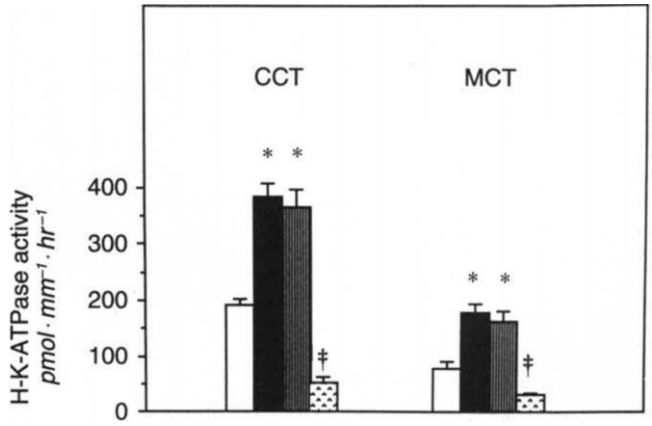

Fig. 3. *The effect of furosemide (F) on H-K-ATPase activity in CCT and MCT in adrenal intact (AI) and adrenalectomized (ADX) rats;* N = *6 in each group*. Symbols are: (■) AI + F; (▨) ADX + F; (▩) ADX + F + V; (□) control. *$P < 0.05$, ‡$P < 0.005$ vs. control animals.

creatinine clearance was affected in the three experimental groups as compared to control. Body weight at the beginning of the study was identical in all four groups, however, at the end of the study body weight in the three experimental groups was significantly lower than in controls.

Alterations in acid-base composition

As compared to control, animals in AI + F group (Fig. 1, black bar), which had the highest aldosterone level and hypokalemia, developed the most profound metabolic alkalosis (HCO_3^- = 36.6 ± 1.2 mEq/liter vs. 22.4 ± 2.1 mEq/liter, respectively, $P < 0.005$). In the ADX + F group (Fig. 1, striped bar), which had normal aldosterone levels and hypokalemia, changes in acid-base status were significantly less severe than in the AI + F group, but the bicarbonate was still significantly greater than control (HCO_3^- = 28.2 ± 1.8 mEq/liter, $P < 0.05$ from control or from AI + F). On the other hand, in the ADX + F + V group (Fig. 1, stippled bar), which also had normal aldosterone and hypokalemia only modest changes in acid-base composition were observed (HCO_3^- = 20.4 ± 1.8 mEq/liter, NS from control).

H-ATPase activity

In the AI + F group (Fig. 2, black bars) which had the highest aldosterone levels, H-ATPase activity was markedly enhanced in both CCT and MCT, but activity in MTAL remained unchanged as compared to control. The percent increase in H-ATPase activity in CCT and MCT averaged 74 ± 7.6% and 104 ± 8.4%, respectively ($P < 0.005$ from their controls). By contrast, in the ADX + F group (Fig. 2, striped bars) which had normal aldosterone levels, H-ATPase activity was similar to control in both CCT and MTAL, while in MCT enzyme activity was slightly but significantly increased (27 ± 3.6% greater than control, $P < 0.05$). In the ADX + F + V group (Fig. 2, stippled bars), H-ATPase activity, again, was unchanged from control in CCT; in MCT, enzyme activity was slightly increased (22 ± 4.2% greater than control, $P < 0.05$). Of interest, H-ATPase activity in the MTAL of the vanadate-treated group was significantly increased (to 40 ± 6.2% of control, $P < 0.05$).

H-K-ATPase activity

In the AI + F group (Fig. 3, black bars), H-K-ATPase activity was markedly increased in CCT and MCT as compared to control (Fig. 3, white bars). The percent increase in H-K-ATPase activity in CCT and MCT averaged 101 ± 12% and 131 ± 21%, respectively ($P < 0.01$). In the ADX + F group (Fig. 3, striped bars), the enzyme activities in CCT and MCT were also very high and were similar to the AI + F group. H-K-ATPase activities in CCT and MCT were enhanced by 92 ± 7% and 113 ± 12%, respectively ($P < 0.01$). In contradistinction, the ADX + F + V rats (Fig. 3, stippled bars) had a marked reduction in H-K-ATPase activity in both CCT and MCT. The percent decrease in the enzyme activity in CCT and MCT as compared to control averaged 72 ± 5%, 60 ± 5%, respectively ($P < 0.005$). H-K-ATPase activity was very low in MTAL (data not shown) in all experimental groups.

Na-K-ATPase activity

In the AI + F group (Fig. 4, black bars), Na-K-ATPase activity in CCT and MCT was markedly stimulated as compared to control (Fig. 4, white bars; to 128 ± 21% and 140 ±

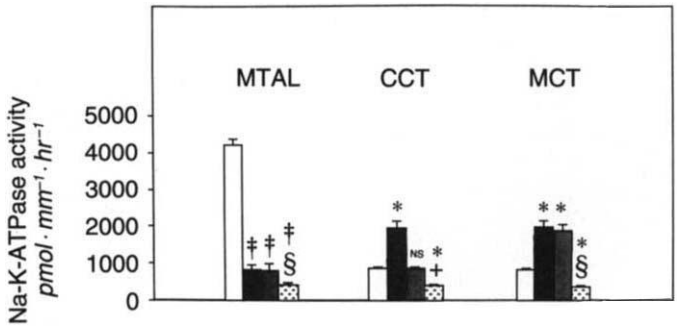

Fig. 4. *The effect of furosemide (F) on Na-K-ATPase activity in MTAL, CCT, and MCT in adrenal intact (AI) and adrenalectomized (ADX) rats;* N = *6 in each group.* Symbols are: (■) AI + F; (▨) ADX + F; (▩) ADX + F + V; (□) control. $*P < 0.01$, $‡P < 0.005$ vs. control animals; $§P < 0.05$ vs. AI + F and vs. ADX + F; $+P < 0.01$ vs. AI + F; NS = not significant vs. control.

21%, respectively, $P < 0.01$). On the other hand, Na-K-ATPase activity in the ADX + F group (Fig. 4, striped bars) was not significantly different from control in CCT. In MCT, Na-K-ATPase activity was stimulated (to 127 ± 20% of control, $P < 0.01$). In the ADX + F + V group (Fig. 4, stippled bars) there was a marked reduction in Na-K-ATPase activity in both CCT and MCT (to 46 ± 4% and 44 ± 6% of control, respectively, $P < 0.01$). In all the experimental groups there was approximately an 80% reduction in Na-K-ATPase activity in MTAL. The percent decrease in the enzyme activity averaged 80 ± 5% in the AI + F group, 81 ± 5% in the ADX + F group, and 90 ± 6% in the ADX + F + V group ($P < 0.005$ vs. control). Na-K-ATPase activity in MTAL, CCT and MCT in the vanadate-treated group was significantly lower than that found in the same nephron fragments of the AI + F and ADX + F groups.

Thus, in the AI + F group which had high aldosterone levels and hypokalemia, severe metabolic alkalosis (HCO_3^-, 36.6 mEq/liter) occurred in association with stimulation of collecting tubule H-ATPase, H-K-ATPase, and Na-K-ATPase activities. In the ADX + F group, which had normal aldosterone levels and hypokalemia, plasma bicarbonate was moderately higher than control (that is, HCO_3^- of 28.2 mEq/liter vs. 22.4 mEq/liter), and this was accompanied by increased H-K-ATPase activity but normal H-ATPase activity. On the other hand, in the ADX + F + V group which had normal aldosterone levels, hypokalemia, and vanadate only modest changes in acid-base composition were observed (HCO_3^-, 20.4 mEq/liter) in association with markedly reduced collecting tubule H-K-ATPase and Na-K-ATPase activities. This group also had increased MTAL H-ATPase activity but its activity in the collecting tubule (CCT and MCT) was unchanged.

Discussion

Hypokalemia is the most common complication of diuretic use [2]. Furosemide can decrease the plasma potassium concentration of hypertensive patients who are not taking potassium supplements by an average of 0.3 mmol/liter in only one week [21]. Studies demonstrate that the major mechanism of the kaliuresis caused by loop diuretics is an increase in distal potassium secretion [22]. Inhibition of potassium reabsorption by the loop of Henle appears to play only a minor role in potassium wastage [23]. Increased distal tubular fluid flow, and increases in aldosterone, catecholamines, and antidiuretic hormone further enhance distal potassium secretion.

Furosemide frequently causes metabolic alkalosis [1–4]. During initiation of therapy, the generation of metabolic alkalosis is primarily caused by increased net acid excretion [24]. However, the maintenance of metabolic alkalosis is due to volume contraction, hypokalemia, mineralocorticoid excess, and continued sodium delivery to the distal nephron [25].

The final regulation of urinary acidification occurs in the collecting tubule [6]. According to current concepts collecting tubule proton secretion is thought to be controlled by two ATPases, the electrogenic H-ATPase and the electroneutral H-K-ATPase [11, 12, 18, 26]. Previous studies have shown that aldosterone stimulates collecting tubule H-ATPase activity while plasma potassium controls H-K-ATPase activity [7–12]. When aldosterone rises and plasma potassium falls, both enzymes are stimulated and metabolic alkalosis occurs. When these two enzymes change in different directions, however, the effect on acid-base composition changes is modest (for example, as might be seen with hypokalemia and decreased aldosterone) [27].

The present studies were conducted to examine the effect of furosemide on acid-base homeostasis and electrolyte composition and to correlate these findings with biochemical data on renal transport in enzymes in rat microdissected MTAL, CCT and MCT. The transport enzymes studied were Na-K-ATPase, H-ATPase, and H-K-ATPase. The studies were performed in both adrenal intact (AI) and hormone replete ADX animals. In the ADX rats the animals were given a fixed physiologic dose of aldosterone to control for the effects of the hormone on proton secretion and potassium homeostasis. All ADX rats were glucocorticoid replete. In the AI + F group, which had high aldosterone levels and hypokalemia, profound metabolic alkalosis occurred in association with stimulation of both collecting tubule H-ATPase and H-K-ATPase. In the ADX + F group, which had normal aldosterone levels and hypokalemia, plasma bicarbonate increased moderately and only H-K-ATPase activity was substantially increased (in CCT and MCT). In this group we also found that MCT H-ATPase activity was slightly increased. In the ADX + F + V group, a group having normal aldosterone levels and hypokalemia, plasma bicarbonate was unchanged as compared to control. In the ADX + F + V animals, collecting tubule H-K-ATPase activity was markedly reduced; a slightly increased H-ATPase activity in MCT was still observed, as noted in the ADX + F group. In a previous study we showed that in adrenalectomized rats given a physiologic dose aldosterone, MCT H-ATPase activity was identical to control [27]. Therefore, the slightly increased MCT H-ATPase activity observed in this study in both the ADX + F and ADX + F + V groups is likely due to the stimulatory effect of furosemide.

In the MTAL, H-ATPase were normal in the AI + F and the ADX + F groups. In the ADX + F + V group, however, H-ATPase activity of MTAL was increased while H-K-ATPase activity was markedly decreased. This stimulatory effect of *in vivo* vanadate on MTAL H-ATPase is similar to our previous work showing stimulation of the enzyme by *in vitro* vanadate [19].

Recent studies suggest that aldosterone plays the major role in controlling Na-K-ATPase activity in CCT while hypokalemia

exerts an aldosterone-independent stimulatory effect on the enzyme in MCT [13–16]. In agreement with these observations, the AI + F group, which had very high aldosterone levels and hypokalemia, had increased Na-K-ATPase activity in both CCT and MCT. In the ADX + F group, animals which had normal aldosterone levels and hypokalemia, Na-K-ATPase was increased, but only in the MCT. Enzyme activity in CCT was identical to control. Vanadate decreased Na-K-ATPase activity in both CCT and MCT in the ADX + F + V group. The fall in Na-K-ATPase was qualitatively, but not quantitatively, similar to that seen when vanadate is administered alone to intact animals [18]. Furosemide decreased MTAL Na-K-ATPase activity in all three experimental groups. Vanadate administration in the ADX + F + V group further depressed MTAL Na-K-ATPase activity as compared to the values seen in the ADX + F group.

The metabolic alkalosis of chronic furosemide administration is associated with stimulation of collecting tubule ATPases. The H-ATPase was stimulated by aldosterone while the H-K-ATPase was activated by hypokalemia. In the CCT, Na-K-ATPase was enhanced by aldosterone while in the MCT, Na-K-ATPase was driven by hypokalemia. In this study, the animals which received vanadate had a normal plasma bicarbonate concentration. We previously showed that chronic vanadate administration causes hypokalemic distal renal tubular acidosis when given in this dose to rats [18]. The difference between the present study and the previous one is the administration of furosemide. As shown in this study, furosemide alone stimulates H-ATPase activity in the MCT. It is possible that this stimulation counteracted the inhibitory effect of vanadate on H-K-ATPase, resulting in "normal" whole kidney acidification. A direct study of urinary acidification is necessary to confirm this suspicion.

Patients with Bartter's syndrome have hypokalemic metabolic alkalosis [28]. It is likely that Bartter's syndrome results from impaired thick ascending limb transport and, therefore, resembles chronic furosemide administration [29]. Our data show that hypokalemia develops in adrenalectomized rats with physiologic aldosterone replacement in association with an increase in fractional potassium excretion. This observation suggests that potassium wastage is due, in part, to impaired potassium absorption in the ascending limb. Increased potassium excretion was seen even after Na-K-ATPase inhibition, a maneuver which should reduce cortical collecting tubule potassium secretion.

We suggest the following hypothesis about Bartter's syndrome. Impaired ascending limb transport causes sodium chloride and potassium loss. The salt loss stimulates aldosterone release. Aldosterone stimulates the H-ATPase enzyme in both CCT and MCT while stimulating the Na-K-ATPase enzyme in CCT. This effect on Na-K-ATPase further stimulates the potassium wastage. Hypokalemia stimulates H-K-ATPase activity in both nephron sites and enhances Na-K-ATPase activity in the MCT. If luminal Na-K-ATPase exists, as proposed by Hayashi and Katz [15], the stimulation of the Na-K-ATPase enzyme would mitigate the potassium losing effect, but acidification would be markedly stimulated. The result would be hypokalemic metabolic alkalosis. The persistence of hypokalemia in patients with Bartter's syndrome following adrenalectomy suggests that decreased thick ascending limb reabsorption is the etiology [30, 31].

In summary, our data are in agreement with an increasing number of reports which suggest that terminal nephron acidification is mediated by two proton ATPases, an H-ATPase and an H-K-ATPase. The former appears to be regulated by aldosterone while the latter responds inversely to plasma potassium concentration. Cortical collecting tubule potassium transport appears mediated by both the Na-K-ATPase and the H-K-ATPase.

Acknowledgments

A portion of this study was presented in November 1992 at the Annual Meeting of the American Society of Nephrology, Baltimore, Maryland. This work was supported in part by grants from the National Institutes of Health, No. R01-DK-36119 and No. R01-DK-36199. Somchai Eiam-Ong is the recipient of a National Kidney Foundation Fellowship Award. The authors thank Ms. Christy Stroud for her typographical assistance and Ms. Betty Lonis for her technical assistance.

Reprint requests to Sandra Sabatini, Ph.D., M.D., Department of Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas 79430, USA.

References

1. Wilcox CS: Diuretics in *The Kidney* (chap 46, 4th ed), edited by Brenner BM, Rector FC Jr, Philadelphia, W.B. Saunders, 1991, p. 2123
2. Suki WN, Eknoyan G: Physiology of diuretic action in *The Kidney: Physiology and Pathophysiology* (Chapt 108, 2nd ed), edited by Seldin DW, Giebisch G, New York, Raven Press, Ltd, 1992, p. 3629
3. Bichet DG, Anderson RJ, Schrier RW: Renal sodium excretion, edematous disorders, and diuretic use in *Renal and Electrolyte Disorders* (4th ed), edited by Schrier RW, Boston, Little, Brown and Co., 1992, p. 1989
4. Quamme GA: Loop diuretics, in *Diuretics: Physiology Pharmacology and Clinical Use* (Chapt 5), edited by Dirks JH, Sutton RAL, Philadelphia, W.B. Saunders, 1986, p. 116
5. Burg M, Stoner L, Cardinal J, Green N: Furosemide effect on isolated perfused tubules. *Am J Physiol* 225(1):119–124, 1973
6. Alpern RJ, Stone DK, Rector FC Jr: Renal acidification mechanisms, in *The Kidney* (chapt 9, 4th ed), edited by Brenner BM, Rector FC Jr, Philadelphia, W.B. Saunders, 1991, p. 318
7. Mujais SK: Effects of aldosterone on rat collecting tubule N-ethylmaleimide-sensitive adenosine triphosphatase. *J Lab Clin Med* 109:34–39, 1987
8. Garg L, Narang N: Effects of aldosterone on NEM-sensitive ATPase in rabbit nephron segments. *Kidney Int* 34:13–17, 1988
9. Khadouri C, Marsy S, Barlet-Bas C, Doucet A: Short-term effect of aldosterone on NEM-sensitive ATPase in rat collecting tubule. *Am J Physiol* 257:F177–F181, 1989
10. Khadouri C, Marsy S, Barlet-Bas C, Doucet A: Effect of adrenalectomy on NEM-sensitive ATPase along rat nephron and on urinary acidification. *Am J Physiol* 253:F495–F499, 1987
11. Doucet A, Marsy S: Characterization of K-ATPase activity in distal nephron stimulation by potassium depletion. *Am J Physiol* 253:F418–F423, 1987
12. Garg LC, Narang N: Ouabain-insensitive K-adenosine triphosphatase in distal nephron segments of the rabbit. *J Clin Invest* 81:1204–1208, 1988
13. Mujais SK, Chekal MA, Jones WJ, Hayslett JP, Katz AI: Modulation of renal sodium-potassium-adenosine triphosphatase by aldosterone. Effect of high physiologic level on enzyme activity in isolated rat and rabbit tubules. *J Clin Invest* 76:170–176, 1985
14. Mernissi GE, Doucet A: Short-term effects of aldosterone and dexamethasone on Na-K-ATPase along the rabbit nephron. *Pflügers Arch* 399:147–151, 1983

15. HAYASHI M, KATZ AI: The kidney in potassium depletion. Na^+-K^+-ATPase activity and [^{3}H] ouabain binding in MCT. *Am J Physiol* 252:F437–F446, 1987
16. IMBERT-TEBOUL M, DOUCET A, MARSY S, SIAUME-PEREZ S: Alterations of enzyme activities along the rat collecting tubule in potassium depletion. *Am J Physiol* 253:F408–F417, 1987
17. KAUFMAN AM, BROD-MILLER C, LEVITT MF, KAHN T: Maintenance of diuretic-induced metabolic alkalosis, in *Diuretics*, edited by PUSCHETT JB, New York, Elsevier Science Publishing Co, 1984, p. 254
18. DAFNIS E, SPOHN M, KURTZMAN NA, SABATINI S: Vanadate causes hypokalemic distal renal tubule acidosis. *Am J Physiol* 262:F449–F453, 1992
19. SABATINI S, KURTZMAN NA: Vanadate stimulates the N-ethylmaleimide-sensitive adenosine triphosphatase in rat nephron. *J Pharmacol Exp Ther* 250:529–533, 1989
20. MARTIN RS, JONES WJ, HAYSLETT JP: Animal model to study the effect of adrenal hormones on epithelial function. *Kidney Int* 24:386–391, 1983
21. MORGAN OB, DAVIDSON C: Hypokalemia and diuretics: An analysis of publications. *Br Med J* 280:905–908, 1980
22. HROPOT M, FOWLER N, KARLMARK B, GIEBISCH G: Tubular action of diuretics: Distal effects on electrolyte transport and acidification. *Kidney Int* 28:477–489, 1985
23. BURG MB: Tubular chloride transport and the mode of action of some diuretics. *Kidney Int* 9:189–197, 1976
24. BATTLE DC: Segmental characterization of defects in collecting tubule acidification. *Kidney Int* 30:546–554, 1986
25. SABATINI S, KURTZMAN NA: Pathophysiology of metabolic alkalosis, in *Therapy of Renal Disease and Related Disorders* (2nd ed), edited by SUKI WN, MASSRY SG, Boston, Martinus Nijhoff Publishing, 1991, pp. 159–175
26. GLUCK S, AL-AWQATI Q: An electrogenic proton-translocating adenosine triphosphatase from bovine kidney medulla. *J Clin Invest* 73:1704–1710, 1984
27. EIAM-ONG S, LONIS B, SABATINI S, KURTZMAN NA: Biochemical basis of metabolic alkalosis. (abstract) *Clin Res* 40:218, 1992
28. BARTTER FC, PRONOVE P, GILL JR JR, MACCARDLE RC: Hyperplasia of the juxtaglomerular complex with hyperaldosteronism and hypokalemic alkalosis. A new syndrome. *Am J Med* 33:811–828, 1962
29. KURTZMAN NA, GUTIERREZ LF: The pathophysiology of Bartter's syndrome. *JAMA* 234:758–759, 1975
30. TRYGSTAD CW, MANGOS JA, BLOODWORTH JM JR: A sibship with Bartter's syndrome: Failure of total adrenalectomy to correct the potassium wasting. *Pediatrics* 44:234–242, 1969
31. TAKAYASU H, ASO Y, NAKAUCHI K, KAWABE K: A case of Bartter's syndrome with surgical treatment followed for four years. *J Clin Endocrinol Metab* 32:842–845, 1971